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A Method for Enhanced Control of Biomass Activity and Distribution in Biofilters

99-253

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ABSTRACT

Long-term performance of vapor-phase bioreactors can be unreliable because of uneven distribution of biomass and microbial activity throughout the bioreactors. One method to improve biomass distribution and maintain high removal efficiencies for continuous long-term use is to operate the bioreactor in a directionally-switching (DS) mode, in which the contaminant inlet is periodically switched between the top and bottom of the reactor column. The objective of this study was to evaluate the effect of DS operation on biomass distribution and activity.

Two identical lab-scale biofilters were operated for 96 days at an inlet toluene concentration of 200 ppm_v and an EBCT of 1 minute. One bioreactor operated in a unidirectional (UD) mode where the air stream was continuously fed to the bottom of the reactor, and the other operated in a DS mode in which the direction of the air stream through the bioreactor was reversed every 3 days. After an initial acclimation period, toluene removal efficiencies of over 99.9% were achieved in both bioreactors for over 40 days of operation. However, toluene removal efficiencies in the UD biofilter declined after 70 days and the pressure drop across the reactor increased quickly, whereas the DS reactor maintained relatively stable operation throughout the same period. The biomass distribution determined by volatile solids and plate counts indicates that the biomass was well distributed in the DS reactor, while excess biomass accumulated in the inlet section of the UD bioreactor. INT (iodonitrotetrazolium chloride) formazan assays were performed to estimate the biomass activity along the length of both bioreactors. These results reveal that biomass activity was more evenly distributed and sustained in the DS bioreactor, but in the UD bioreactor most of the bioactivity was confined to the front half of the bed.

INTRODUCTION

Vapor-phase bioreactors have been used primarily to treat a variety of odorous compounds in contaminated air streams, and their application is currently being expanded to soil vapor extraction and industrial waste gases contaminated with low to moderate concentrations of volatile organic compounds (VOCs). Biofilters are the simplest type of vapor-phase bioreactor, in which the contaminants are absorbed into a stationary liquid biofilm phase and degraded. Several studies have demonstrated that biofilters packed with compost or artificial media can successfully degrade VOCs as well as odorous compounds [1, 2, 3, 4]. Biofilters provide high gas/liquid surface areas that minimize mass transfer limitation. In addition, biofilters are easy to operate and can provide a cost-effective alternative to traditional treatment methods such as scrubbing and thermal incineration [1, 5]. Long-term performance of biofilters, however, can be

unreliable due to clogging and inactivation of the bed materials [5, 6]. These problems stem from an uneven distribution of excess biomass and loss of biomass activity in bioreactors subjected to moderate pollutant loadings. These bioreactor problems have not been well defined, and the role of biomass activity, in particular, has been neglected in most bioreactor studies.

Several different methods have been proposed to prevent clogging in biotrickling filters including nutrient control and mechanical biomass removal. Providing an adequate but minimal amount of essential nutrients may prevent excess biomass growth as well as maintain biodegradation capacity. Since nitrogen is one of the major cell constituents, nitrogen limitation may be an effective method for the control of biomass accumulation. However, nitrogen limitation results in a significant decrease in pollutant removal capacity, whereas potassium limitation was found to be a more effective solution [6, 7]. Another biomass control method is to remove excess biomass mechanically. Smith et al. [8] found that frequent (i.e., two times per week) backwashing with full fluidization at a recirculation rate of 190 m/h effectively removed excess biomass accumulation. Another approach proposed by Kinney [4] is directionally-switching (DS) operation, in which the contaminant inlet is periodically switched between the top and bottom of the reactor column. This investigation found that a biofilter operated in the DS mode maintained high toluene removal efficiencies and stable performance without system failure for over 4 months. However, several questions remain to be addressed such as the spatial distribution of biomass and activity throughout DS bioreactors.

While biomass accumulation is an important issue, biomass activity is also a key parameter for successful operation and control of biofilters. Biofilter performance and elimination capacity are a function of microbial activity, but few studies have been investigated the role of microbial activity in vapor-phase bioreactors. Moreover, changes in microbial activity during bioreactor operation are also neglected. Since all viable microorganisms have active electron transport systems consisting of a series of dehydrogenase enzymes, measurements of dehydrogenase activity can be employed to estimate microbial viability and respiratory activity in biomass samples [9]. Tetrazolium salts such as 2,3,5-triphenyltetrazolium chloride (TTC), 5-cyano-2,3-ditolyltetrazolium chloride (CTC) and 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (INT) are commonly used as an indicator for the dehydrogenase activity [10, 11]. Among the three tetrazolium salts, the INT-formazan assay is the most suitable for determining the activity of biomass attached on a solid support media because it is very stable and easy to extract for quantification purposes and it is less sensitive to oxygen [9]. Thus, the INT-formazan assay may be useful as a tool to determine biofilm activity and provide a fundamental understandings of the biofiltration process.

The objectives of the research were to: (1) evaluate a directionally-switching (DS) biofilter subjected to a high toluene loading and compare the performance of a DS biofilter to that of the unidirectional (UD) biofilter; (2) investigate the effect of each operation mode on biomass accumulation and activity, and (3) determine biofilm characteristics affecting toluene degradation and system performance.

MATERIAL AND METHODS

Bioreactor Configuration, Operation and Start-up

Two lab-scale biofilters, each consisting of a stainless steel column with an inner diameter of 16.2 cm, were operated as shown in Figure 1. Each reactor column was divided into six sections, and the middle four sections each contained porous silicate pellets (Celite® R-635, Lompoc, CA) to a depth of 25 cm, resulting in an overall packed bed height of 100 cm. The packing media was supported by perforated stainless steel plates located at the bottom of each section and each section was separated by a 8 cm gas sampling plenum. Each biofilter had 5 gas sampling ports and 4 media sampling ports along the column.

The compressed air supply to each biofilter column was split into two streams. Research grade pure toluene was injected into one air stream using a syringe pump (Model 200, KD Scientific, MA). A nebulizer (Heart®, Tucson, AZ) generated a nutrient laden aerosol in the other air stream. The two air streams were combined in a mixing chamber. The combined air stream with a target toluene concentration of 200 ppmv was supplied to each biofilter at a flow rate of 19.8 L/min for an empty bed residence time of 1 minute. One bioreactor operated in a UD mode where the air stream was continuously fed to the bottom of the reactor, and the other operated in a DS mode in which the direction of air stream through the bioreactor was reversed every 3 days. Other operating parameters such as air flowrate, inlet toluene concentration and nutrient supply were maintained identically in each biofilter throughout the 96 day experimental period.

An inoculum solution originally seeded with leachate from a biofilter column operated in our laboratory was cultured with toluene vapor. Prior to start-up, the inoculum was recirculated slowly for 6 hours throughout each bioreactor. After draining the inoculum from each column, the contaminated air stream described above was supplied to each bioreactor.

Water and Nutrient Supply

A fine aerosol generated by a nebulizer was fed to each bioreactor to supply moisture and nutrients that are essential for microbial growth and activity. The nebulizer atomized a nutrient-containing buffer solution to provide each bioreactor with about 400 mL/day of nutrient solution. The nutrient solution used for this experiment consisted of a hydrocarbon minimal media (HCMM) slightly modified from the composition described elsewhere [12] (see Table 1). In order to investigate the effects of nutrients on performance, the phosphate, ammonia and nitrate concentrations were modified four times over the course of the experiments, while the trace metal concentration was held constant. After start-up with the HCMM solution, the nitrate concentration in the HCMM solution was doubled between days 8 and 13. On day 13, the HCMM solution was switched to tap water until day 17, when the tap water was replaced with a HCMM solution containing nitrate at three times the original concentration. On day 24, the composition of the nutrient solution was changed for a final time and this composition was used for the remainder of the experiment (see Table 1).

Analytical Methods for Gas and Packing Media Samples

To determine overall toluene removal efficiencies and toluene profiles along the column, gas samples were collected daily with 0.5-mL gas-tight syringes (Hamilton 1700 series) from each sampling port. The samples were immediately analyzed using a gas chromatograph (Hewlett Packard 6890) equipped with a flame ionization detector (GC/FID) and a 30 m HP-5 capillary column.

Packing media samples were periodically collected and analyzed for volatile solids (VS) and total heterotrophic cell counts. The volatile solids were measured by weight loss following ignition of the media samples at 550°C. Bacterial populations were enumerated by spreading the diluted samples on R2A agar (Difco, Detroit, MI) plates. Colony forming units (CFUs) were calculated by averaging colony numbers in triplicate samples after a 5-day incubation period. One set of blank plates was always prepared as a quality assurance check.

The INT assay procedure used in this study was a modification of procedures employed by Anderson et al. [10] and Blenkinsopp and Lock [11]. Each pellet sample was placed in 10 mL of Trizma™ (Fisher, Fair Lawn, NJ) extraction media (6.35 g/L of Trizma HCl, 1.18 g/L of Trizma Base, 9.0 g/L of NaCl) and then vortexed for 20 seconds and sonicated for 20 seconds. 2 mL of 0.02%(w/v) INT (Aldrich, Milwaukee, WI) solution was added, and the suspension was mixed and incubated at room temperature for four hours in the absence of light due to light sensitivity of INT. One mL of 37% formaldehyde was then added to stop the reaction. The mixture was centrifuged at 2100g for 10 minutes, and then the supernatant was carefully decanted. The INT-formazan crystal (dark red color) was disrupted by sonication and extracted with 5 to 12 mL of methanol, and the extraction was continued in the dark for 15 minutes. Color development of the extraction was measured on a spectrophotometer at 480 nm against a methanol blank. Three INT-formazan (Aldrich, Milwaukee, WI) standards prepared in methanol were used to prepare a standard curve. Controls were provided by adding 1 mL of 37% formaldehyde to biomass samples to stop all bioactivity before INT treatment, and the absorbency of the controls was deducted from that of the biomass samples.

RESULTS AND DISCUSSION

Biofilter Performance

Biofilm Nutrient Requirements

The effect of nutrient supply on overall toluene removal efficiency and biodegradation reaction kinetics was determined over the first 24 days of the study. By day 17 of operation, the overall toluene removal efficiency in both biofilters (DS and UD) fluctuated between 40% and 90% depending on the amount of total nitrogen supplied. It was observed that the greater the total nitrogen supply, the higher the overall removal efficiency. Both biofilters were very sensitive to nitrogen levels and were operating in a nutrient (nitrogen)–limited condition. In addition, as shown in Figure 2, the toluene profiles along the columns of both biofilters were found to be linear functions of bed depth, indicating zero order biodegradation kinetics.

After increasing the total nitrogen (ammonia + nitrate) content of the nutrient solution by three times on day 17, the overall toluene removal efficiency in each bioreactor immediately increased to greater than 99% and first order exponential removal of toluene throughout each biofilter was observed (see Figure 3). These results verify that both biofilters required a high concentration of total nitrogen to maintain microbial growth and biodegradation. In addition, microbial growth shifted from maintenance metabolism under nutrient limitation to an active growth phase with exponential toluene removal under nutrient-rich conditions.

It should be noted that only a fraction of the total amount of nitrogen supplied to the biofilters may be directly available to microorganisms in the biofilm. Gribbins and Loehr [13] suggested that a threshold amount of soluble nitrogen of 1 g N/kg packing media was required for a compost biofilter at a toluene loading of 30 g/m^3-hr. However, few studies have been performed on nitrogen availability for biofilters packed with artificial media. Better information about nitrogen requirements for different types of packing media and microorganisms is required to enhance system start-up and maintain stable biofilter performance.

Exponential Toluene Removal and Biomass Activity Loss

After the nutrient concentrations were increased in the aerosol feed on day 17, toluene removal efficiencies of >99% were observed for the next 45 days in the UD biofilter and for the remainder of the 96 day study period in the DS biofilter. The toluene degradation profiles indicate that each bioreactor progressed through a period of exponential toluene removal followed by a declining biomass activity phase and less efficient toluene removal. In the DS biofilter, the toluene degradation profiles showed first order exponential curves along the biofilter column for the initial 35 days of the study, followed by a slow loss of biodegradation activity in the biofilm for the remainder of the experiment. The duration of the exponential removal phase in the UD biofilter was shorter than that of the DS operation (i.e., about 15 days), and biodegradation activity loss proceeded faster from the inlet section to the outlet section of the biofilter. The exponential toluene removal phase and the biomass activity loss phase are each discussed in more detail below.

During the exponential toluene removal phase, there was no significant difference in toluene degradation profiles. The toluene profiles indicate that greater than 90% of the inlet toluene was removed in the front half of bed and the final 10% of toluene was degraded in the back half of bed (see toluene profiles in Figure 3, day 35). After the exponential toluene removal phase, the toluene degrading capacity began to decrease in the UD biofilter. As shown in Figure 4, the front half of bed in the UD biofilter lost 50% of its toluene degrading capacity by day 60, while the back half of the bed still actively degraded toluene corresponding to an overall removal efficiency of >99%. In contrast, the DS biofilter maintained exponential toluene removal along the column throughout the same period. Even though the overall removal efficiencies were both >99%, the biofilter sections where active biodegradation took place were quite different. Since the UD biofilter needed the entire column to achieve an overall efficiency of >99%, a step increase in the inlet toluene loading resulted in toluene breakthrough. No such breakthrough was observed in the DS system (data not shown). These results indicate that one of the potential advantages of DS operation over conventional operation (i.e., UD operation) is that the active pollutant-degrading capacity is maintained more evenly throughout the entire bioreactor.

Toluene Breakthrough

The activity loss in the UD biofilter progressed quickly from the inlet section to the outlet section of the biofilter column until eventually the UD biofilter lost most of its toluene degradation capacity. In the DS biofilter, the degradation capacity also slowly decreased from the inlet section of the reactor from day 60 to the end of the study; however, significant breakthrough (an overall removal efficiency of <95%) never occurred in the DS biofilter. For comparison, the entire column of the UD biofilter degraded only 35% of the inlet toluene on day 91, whereas >99% of the inlet toluene was removed in the DS biofilter on the same day (see Figure 5).

Pressure drop across the biofilters was one possible reason for the loss of toluene degradation capacity. The pressure drop rapidly increased with toluene degradation capacity loss in the UD biofilter. When the breakthrough occurred in the UD biofilter, the pressure drop across the column was greater than 5 inches of water/m. The high pressure drop was likely due to excess biomass build-up and free water hold-up in the packing media (see discussion below). In the DS biofilter, pressure drop did not exceed 3 inches of water/m over the entire period of operation.

Biomass and Activity Changes

Biomass Accumulation and Distribution

During the nutrient limitation phase when toluene degradation took place throughout each biofilter column, the biomass increased evenly along the length of each column. After exponential toluene removal profiles were observed, biomass accumulated mostly in the inlet bioreactor section(s) where the carbon supply was most abundant. The biomass amount as measured by volatile solids increased rapidly in the inlet section of the UD biofilter, while in the DS biofilter, biomass accumulation primarily took place in both inlet sections of the column. Figure 6 shows the biomass profiles along the DS and the UD biofilter column, respectively, as measured by VSs on day 75. The biomass profile of the DS operation follows a hyperbolic shape with twice as much biomass in both ends of the column as in the middle sections. In contrast, the biomass in the bottom section of the UD biofilter was 9 times greater than that in the outlet section. These biomass distribution profiles verify that biomass was more evenly distributed in the DS biofilter than in the UD biofilter.

The biomass distribution as determined by plate counting revealed the same biomass distribution trends as the volatile solid results. Figure 7 shows the total heterotrophic cell count profiles for day 75 normalized by the weight of packing material. These results indicate that the microbial density in the inlet section of the UD biofilter was about 2 orders of magnitude higher than that in the outlet section, while the maximum difference between the bacterial populations along the DS biofilter column was within 1 order of magnitude. Since the heterotrophic microbial population density counts include only those bacteria which are alive and can form colonies on the R2A agar plates, these results indicate that more viable cells were present in the inlet section(s) of both biofilters. These microbial density results also confirm the uneven distribution of biomass in the UD biofilter and the more even distribution of biomass in the DS biofilter.

Biomass Activity

After the biofilter nutrient requirements were met, the microbial activity as determined by the INT assays increased in the inlet biofilter section(s). Like the biomass distribution results, the

INT activity profiles determined on day 33 show that the inlet of the UD biofilter had the highest respiratory activity, while almost no microbial activity was observed in the back half of bed in the UD biofilter (see Figure 8). Interestingly enough, the respiratory activity in the outlet section of the DS biofilter was higher than that in the second section, even though the outlet section received no toluene (i.e., no external carbon source) on day 33. Because INT reduction is proportional to the amount of active enzymes in the electron transport chain of microorganisms, this result suggests that the biomass in the outlet section was fed with internal carbon sources including cell storage materials and other organic constituents in the biofilm. The INT activity is, therefore, a function of not only the primary carbon source supplied but also other carbon sources which have accumulated in the biomass. These results also indicate that the biomass activity was more evenly distributed and sustained in DS operation as compared to UD operation.

CONCLUSIONS

One DS and one UD biofilter were operated at a toluene loading rate of 45.8 g/m^3-hr for a period of 96 days. The DS system performed better with respect to maintaining stable long-term bioreactor performance. The greatest advantage of DS operation was that biomass accumulated more evenly across the biofilter and higher microbial activity was maintained throughout the column. In the UD biofilter, most of the biomass accumulation and activity was confined to the front half of bed. This skewed distribution caused a high pressure drop and loss of biomass activity and eventually resulted in toluene breakthrough.

Even with a steady toluene inlet loading, biofilter performance in both the UD and DS system continually changed over the course of the study. This dynamic response was initially due to nutrient limitation and later due to loss of biomass activity. The nutrient limitation was due to an inadequate supply of nitrogen which detrimentally affected the overall toluene removal efficiency and biodegradation kinetics. The loss of biomass activity in overall bioreactor performance in the UD system were caused by excess biomass accumulation and high pressure drop. In order to maintain stable bioreactor performance, biomass accumulation and activity control methods such as DS operation are needed and careful monitoring of the biomass within the system is required.

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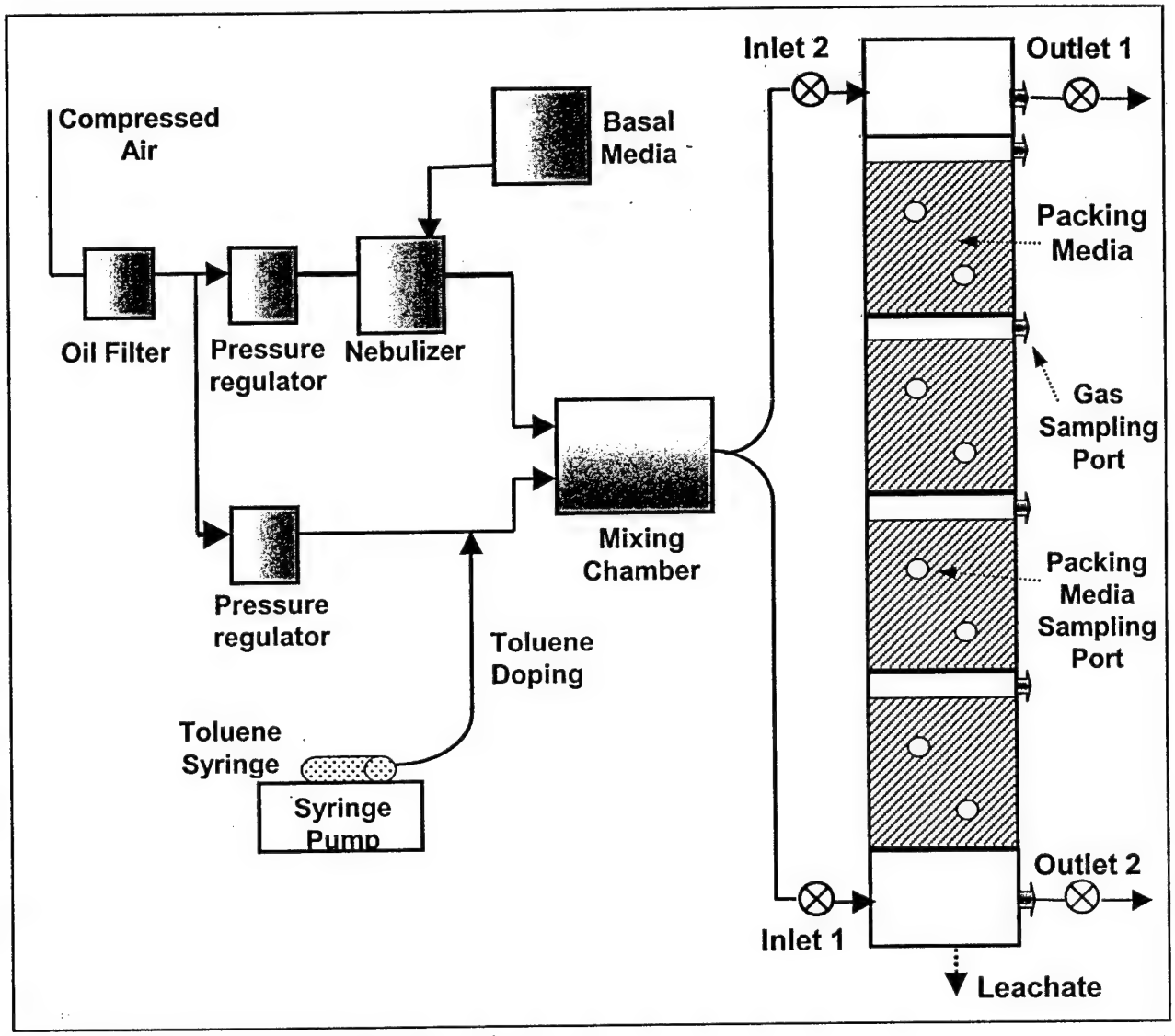

Figure 1. Schematic of the vapor-phase bioreactor.

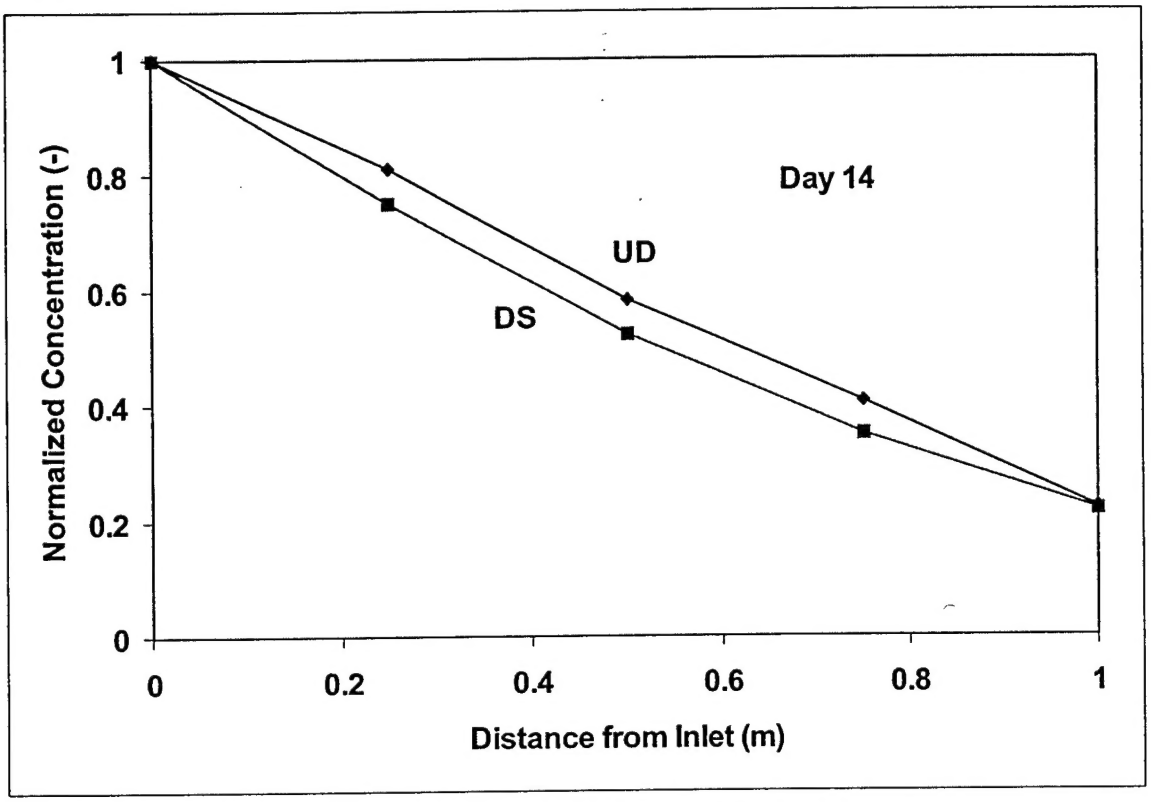

Figure 2. Toluene profiles along the DS and UD bioreactor on day 14.

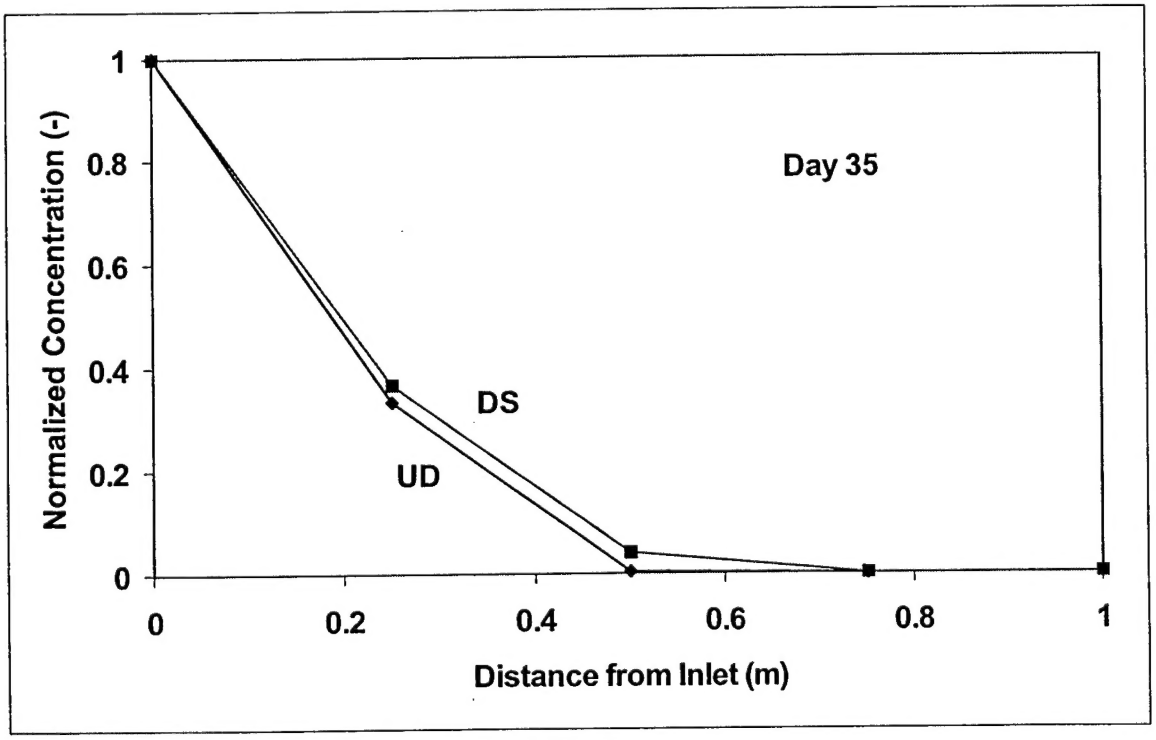

Figure 3. Toluene profiles along the DS and UD bioreactor on day 35.

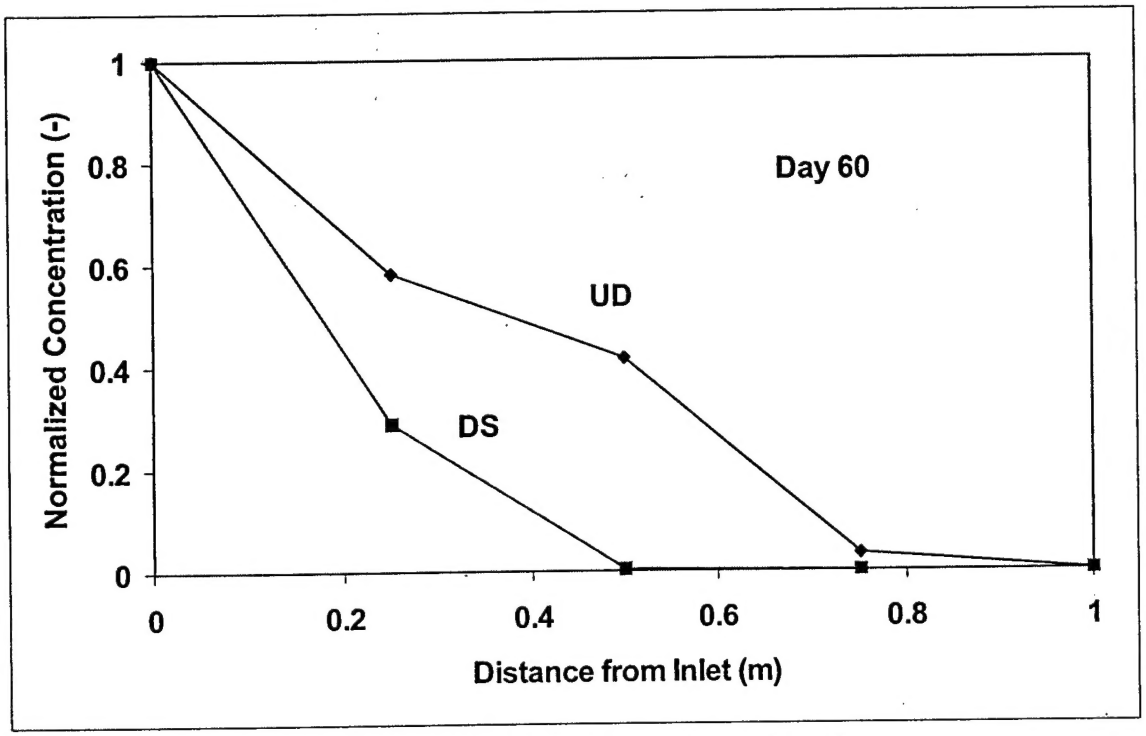

Figure 4. Toluene profiles along the DS and UD bioreactor on day 60.

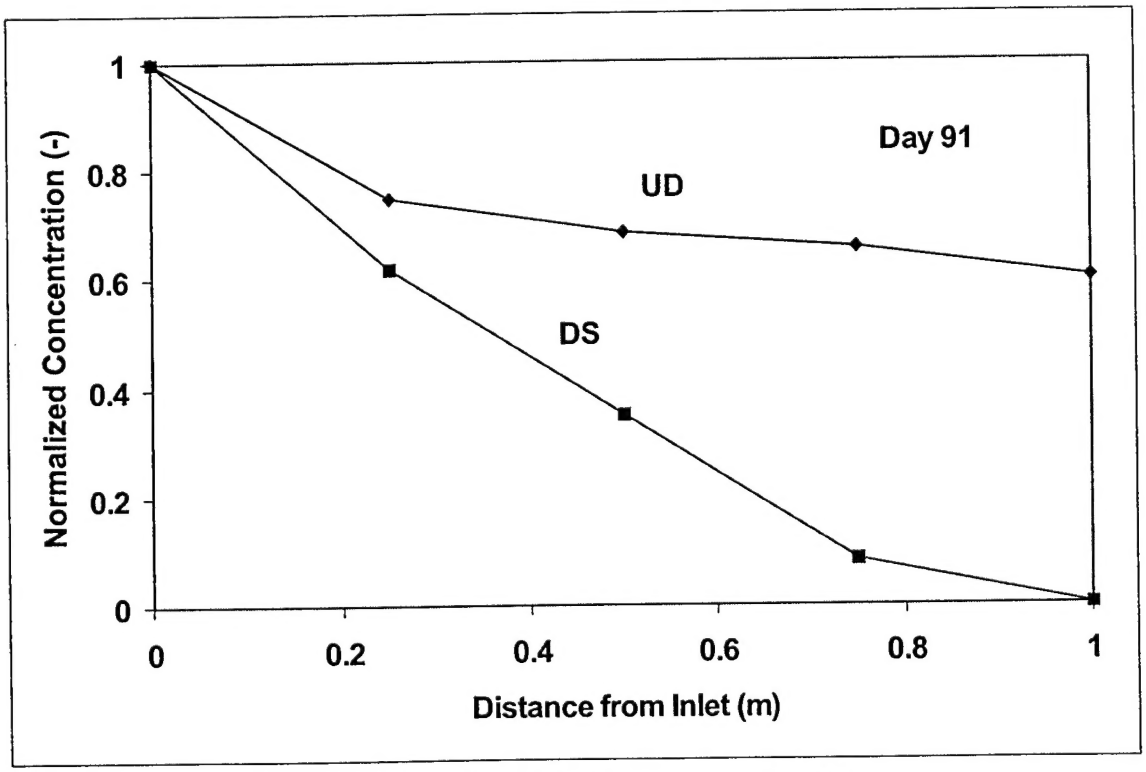

Figure 5. Toluene profiles along the DS and UD bioreactor on day 91.

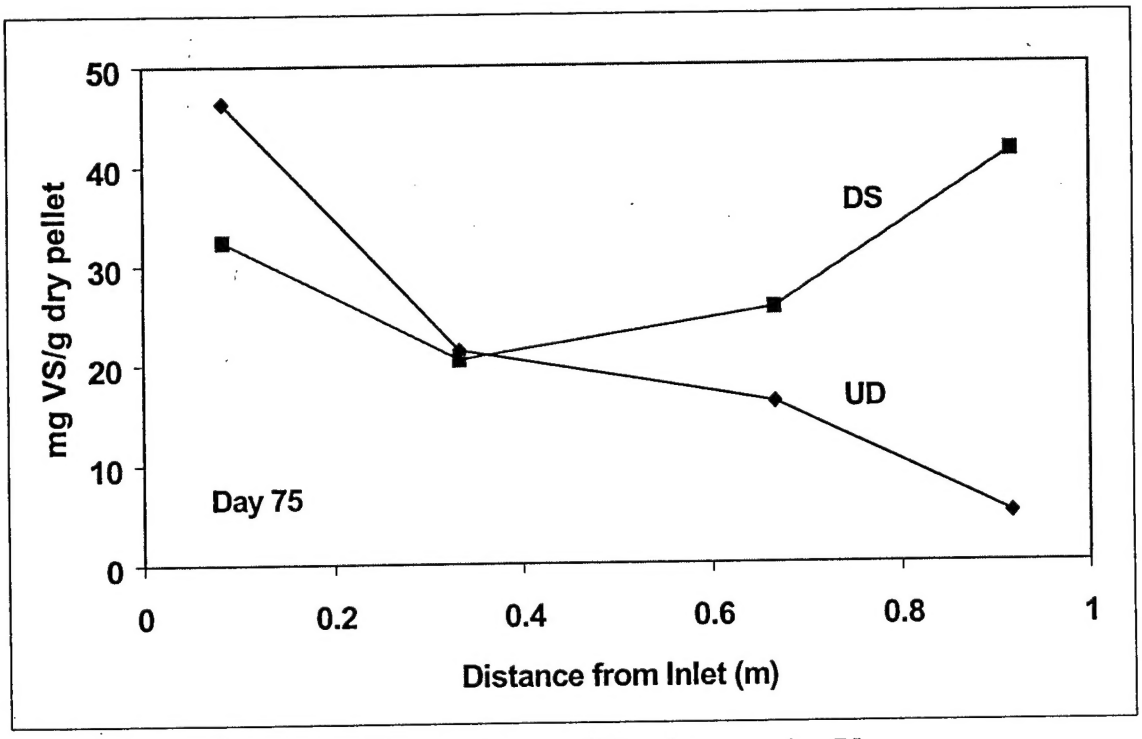

Figure 6. Biomass distribution along the DS and UD column on day 75.

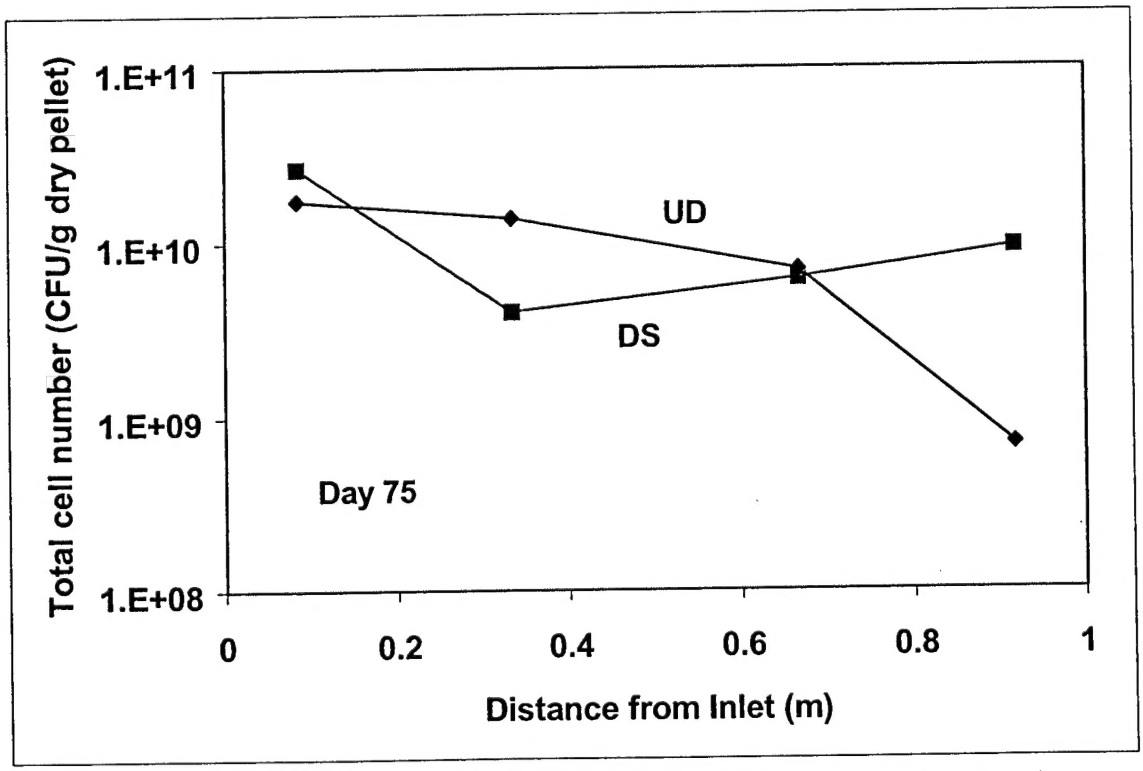

Figure 7. Distribution of total heterotrophic microbial population as determined by plate counting on R2A agar media on day 75.

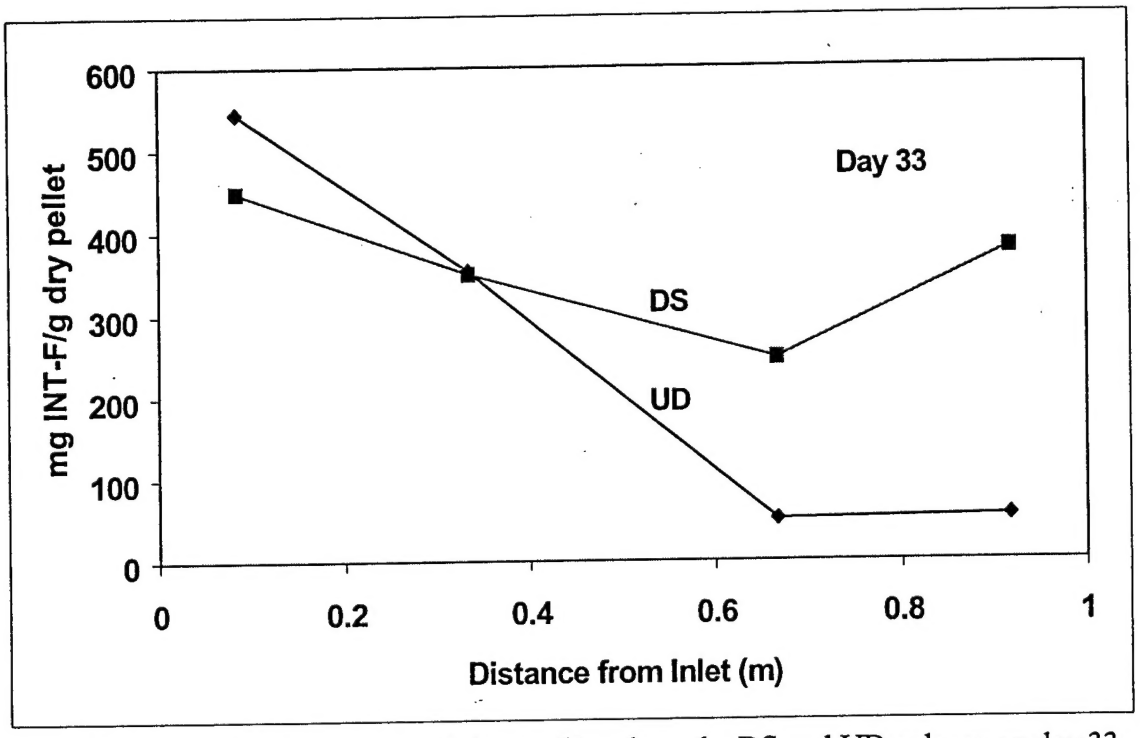

Figure 8. Dehydrogenase activity profiles along the DS and UD column on day 33.

Table 1. Compositions of the nutrient feed solution (HCMM)

Component	Concentration M	Concentration g/L	Component	Concentration mg/L
Primary Nutrients in the Initial Solution			**Trace Metals**	
KH_2PO_4	0.01	1.36	$MgSO_4 \cdot 7H_2O$	0.05
Na_2HPO_4	0.01	1.42	$CaCl_2 \cdot 2H_2O$	0.0147
$(NH_4)_2SO_2$	0.0038	0.5	H_3BO_3	2.86
KNO_3	0.03	3.03	$MnSO_4 \cdot H_2O$	1.54
Primary Nutrients in the Final Solution			$FeSO_4 \cdot 7H_2O$	2.50
KH_2PO_4	0.02	2.72	$CuCl_2 \cdot 2H_2O$	0.027
Na_2HPO_4	0.01	1.42	$ZnSO_4 \cdot 7H_2O$	0.044
$(NH_4)_2HPO_4$	0.01	1.32	$CoCl_2 \cdot 6H_2O$	0.041
KNO_3	0.10	10.1	$Na_2MoO_4 \cdot 2H_2O$	0.025
			$NiCl_2 \cdot 6H_2O$	0.020